LAIDLOMYCIN PHENYLCARBAMATE, A SEMISYNTHETIC POLYETHER ANTIBIOTIC†

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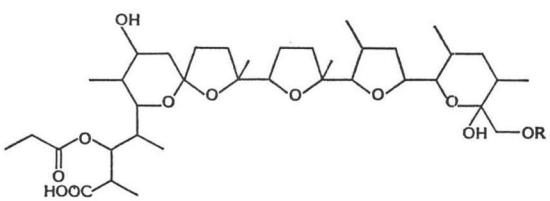
(Received for publication July 28, 1986)

We have previously reported that monoacylation of the antibiotic laidlomycin (**1**) at the C-26 hydroxyl group led to derivatives with enhanced activity toward favorably altering rumen fermentation and preventing avian coccidiosis.[1] Subsequent stability studies on the C-26 propionate ester of laidlomycin indicated that the ester group was somewhat labile either in solution or in a soybean meal feed premix at slightly elevated temperatures (60°C). From a number of other derivatives, the C-26 phenylcarbamate **2** was identified as an agent with greatly improved stability and outstanding activity in screens for identifying manipulators of rumen fermentation and anticoccidial agents.[2] It is interesting to note that 2-phenethylcarbamates of monensins A and B have been isolated as natural products[3,4] and that semisynthetic monensin carbamates have been reported to have antiparasitic activity[5] and to be ten times more active than monensin against *Candida albicans* and *Penicillium digitatum*.[6]



1 R = H
2 R = CONHPh

Laidlomycin phenylcarbamate (**2**) was prepared by treatment of laidlomycin with phenyl isocyanate in pyridine. ^{13}C NMR spectroscopy confirmed that derivatization had occurred at the C-26 hydroxyl group (Table 1). A slight downfield shift of C-26 (0.34 ppm) and C-25 (1.3 ppm) compared to underivatized laidlomycin was observed, while the chemical shift of C-7 was virtually unchanged analogous to the shifts found for the C-26 esters of laidlomycin.[1]

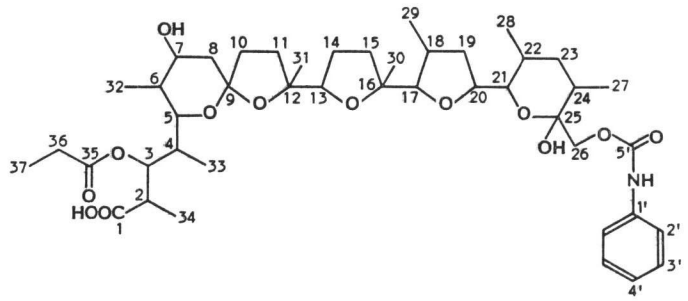
Compound **2** was compared with laidlomycin in rumen fermentation models[1] in which the ability of test compounds to increase propionic acid production and to decrease lactic acid production, both desirable attributes for rumen modifiers,[7] was determined. Data in Table 2 indicate that **2** was significantly more active than laidlomycin in terms of positively influencing both of the test parameters. Thus, **2** increased propionic acid production 21~33% while decreasing lactic acid to 13~21% of control over a dose range of 2.50~20 μg/ml. Laidlomycin, on the other hand, gave an increase of only 9% in propionic acid production at the highest dose (20 μg/ml) and afforded a maximal decrease in lactic acid of only 81% of control. Statistical analysis over the entire concentration range indicated that **2** was more potent than laidlomycin ($P<0.05$) in both assays.

When tested as an anticoccidial agent in chicks, phenylcarbamate **2** also proved to be efficacious at 99~165 mg/kg of feed (Table 3). When compared with data on laidlomycin from the previous study,[1] **2** was considerably more active than laidlomycin in terms of both mean weight gain and lesion score.

In conclusion, the semisynthetic phenylcarbamate of laidlomycin (**2**) is considerably more active than the parent antibiotic in both *in vitro* ruminant growth promotant and *in vivo* chick coccidiosis screens. This compound is also considerably more stable ($T_{1/2}$ of 784 hours vs. 11 hours for laidlomycin propionate, methanol-water, acetate buffer, 60°C) than the previously reported esters.[1] This latter consideration would make it appear very likely that the activity of **2** is inherent in the compound itself as opposed to being a pro-drug which serves to deliver the parent antibiotic to the required site of action.

† Contribution No. 725 from the Institute of Organic Chemistry.

Table 1. ^{13}C NMR data for laidlomycin phenylcarbamate (**2**)[a].



Carbon No.	δ[b]	Carbon No.	δ[b]	Carbon No.	δ[b]
1	180.78	15	30.93	29	15.25
2	43.64	16	84.14	30	23.30
3	75.88	17	86.08	31	28.08
4	38.98	18	35.61	32	9.96
5	67.87	19	32.39	33	10.78
6	34.67	20	77.65	34	16.81
7	70.66	21	75.12	35	173.83
8	34.05	22	32.21	36	27.93
9	107.84	23	36.33	37	9.33
10	39.11	24	35.76	1′	139.07
11	33.79	25	96.84	2′	118.39
12	85.40	26	67.24	3′	128.77
13	82.11	27	16.40	4′	122.58
14	26.93	28	17.63	5′	153.93

[a] δ values in $CDCl_3$, D_2O, K_2CO_3.
[b] For details of assignments see ref 1.

Table 2. Activity of laidlomycin and laidlomycin phenylcarbamate toward altering rumen fermentation *in vitro*.

Concentration of compound (μg/ml)[b]	Propionic acid production (% of control)[a]		Lactic acid production (% of control)[a]	
	Laidlomycin	Laidlomycin phenylcarbamate	Laidlomycin	Laidlomycin phenylcarbamate
0	100	100	100	100
0.31	99	107	101	102
0.62	98	110	110	99
1.25	98	118	94	56
2.50	102	121	115	21
5.00	104	126	95	13
10.00	108	127	77	14
20.00	109	133	81	13

[a] Numbers are propionic acid or lactic acid production expressed as a percentage of control incubations containing no experimental compound. Therefore the amount of either acid produced in the control incubation would be expressed as 100%. Pooled standard errors obtained in any given experiment typically were less than 4% of control incubations.
[b] Represents the concentration of the test compound in the incubation media.

Table 3. Efficacy of laidlomycin phenylcarbamate as an anticoccidial agent for chicks.

Variable	Concentration of laidlomycin phenylcarbamate (mg/kg of feed)				
	0	99	121	143	165
Starting weight (kg)[a]	2.32	2.32	2.25	2.39	2.32
Final weight (kg)	3.04	3.86	3.72	3.97	4.21
Gain (kg/7 days)	0.72	1.54	1.47	1.58	1.89
Feed consumption (kg/7 days)	3.00	3.40	3.19	3.46	3.47
Feed efficacy (feed/gain)	4.18	2.21	2.16	2.19	1.84
Lesion scores[b]					
Upper intestine	2.83	1.20	1.23	0.50	0.66
Middle intestine	2.87	1.43	1.57	1.47	0.70

[a] Experimental unit was the cage containing 12 chicks.
[b] Scores are on a scale of 0 to 4 which range from least to most severe.

Experimental

^{13}C NMR spectra were determined using a Bruker WM-300 (75.475 MHz) spectrometer as previously described.[1] Silica gel for column chromatography was that of Merck (Darmstadt), 70~230 mesh. Microanalyses were performed by Syntex Analytical Research.

Laidlomycin Phenylcarbamate (2)

Phenyl isocyanate (2.38 g, 20 mmol) was added to a solution of laidlomycin (14 g, 20 mmol) in 100 ml of pyridine. The mixture was heated in an oil bath at 70°C for 30 minutes. The mixture was cooled, diluted with dichloromethane, washed with 5% aqueous hydrochloric acid and water, and evaporated. The residue was purified by silica gel chromatography (75% ether hexane, 0.01% formic acid eluent) to afford 10 g of laidlomycin phenylcarbamate as an amorphous solid which melted at 88~90°C.

Anal Calcd for $C_{44}H_{67}NO_{13}$:
C 64.60, H 8.26, N 1.71.
Found: C 64.28, H 8.18, N 1.52.

Rumen Fluid Incubations

These were carried out using the methodology previously described.[1]

Testing for Anticoccidial Activity

Broiler chicks were reared, 12 per cage in wirefloored battery cages under conditions which prevented extraneous coccidial infections. All chicks received a non-medicated starter diet *ad libitum* until 8 days of age. From 8 days of age until the end of the study, chicks received the test diets which contained either 0, 99, 121, 143 or 165 mg of laidlomycin phenylcarbamate per kg of feed. Three replicate pens per treatment were used for all levels of experimental compounds tested.

At 10 days of age, birds were orally challenged with 200,000 oocycts of the coccidia, *Eimeria acervulina*, and 20,000 oocysts of *Eimeria maxima* per bird. The birds were weighed immediately preceding the coccidial challenge and again 7 days later. At 7 days following coccidial challenge, all birds were killed and lesions in the upper and middle sections of the intestinal tracts of six preselected birds per cage were scored on a scale of 0 to 4 based upon their number and severity. Feed present at the time of coccidial challenge and remaining 7 days later was used to determine the effects of compounds upon total feed consumption and feed efficiency.

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